TRANSLATION

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
SAP-717-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2004/017959	02.12.2004	02.12.2003

International Patent Classification (IPC) or national classification and IPC

C07K16/44, C12N5/10, C12N15/02, C12P21/08, G01N33/53

Applicant

ADVANCED LIFE SCIENCE INSTITUTE, INC.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))

      _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/JP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/017959

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed

☐ the translation of the international application into _____ , which is the language of a translation furnished for the purposes of:

☐ international search (Rule 12.3(a) and 23.1(b))

☐ publication of the international application (Rule 12.4(a))

☐ international preliminary examination (Rule 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-18 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

nos. 1,8-11 as originally filed/furnished

nos.* _____ as amended (together with any statement) under Article 19

nos.* 5-7 received by this Authority on 24.02.2006

nos.* _____ received by this Authority on _____

☒ the drawings:

sheets 1-15 as originally filed/furnished

sheets* _____ received by this Authority on _____

sheets* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☒ the claims, nos. 2-4

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☒ the claims, nos. 1,9,10

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/017959

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees the applicant has, within the applicable time limit:
   - ☐ restricted the claims
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☐ neither restricted the claims nor paid additional fees

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:
   - ☐ complied with
   - ☒ not complied with for the following reasons:

The feature that is common to claims 1 and 5 to 11 is the "anti-methyllysine antibodies that specifically recognize methyllysine but are not capable of recognizing lysine."

However, "anti-methyllysine antibodies that specifically recognize methyllysine but are not capable of recognizing lysine" are also disclosed in the document K. PETHE et al. (Proc. Natl. Acad. Sci. USA, (2002), Vol. 99, No. 16, pages 10759 to 10764), which is cited in the international search report, and thus it is apparent that said antibodies are not novel.

[Refer to the Supplemental Box]

4. Consequently, this report has been established in respect of the following parts of the international application:
   - ☐ all parts
   - ☐ the parts relating to claims Nos. _____

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/017959

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	7-10	YES
	Claims	1, 5, 6, 11	NO
Inventive step (IS)	Claims		YES
	Claims	1, 5-11	NO
Industrial applicability (IA)	Claims	1, 5-11	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Document 1: WO 2002/18418 A1 (University Of Virginia Patent Foundation), 07 March 2002

Document 2: K. PETHE et al., Proc. Natl. Acad. Sci. USA, (2002), Vol. 99, No. 16, pages 10759 to 10764

Document 3: Product Datasheet for ab7766, 2002, [online], ABCAM Ltd.

The following document is newly cited in the written opinion and the international preliminary examination report drafted by the International Preliminary Examining Authority

Document 4: U. P. STEINBRECHER et al., J. Lipid Res., 1984, Vol. 25, pages 1109 to 1116

(A)

Document 1 discloses the anti-methyllysine antibody (Methyl(K4)H3) and the anti-methyllysine antibody (Methyl(K9)H3), which specifically recognize methyllysine but do not recognize lysine; indicates that anti-methyllysine antibody (Methyl(K4)H3) and the like are polyclonal antibodies; and discloses a method for

detecting methylated proteins by means of said anti-methyllysine antibodies (in particular, refer to fig. 3).

(B)

Document 2 presents the monoclonal anti-methyllysine antibody (4057D2), which specifically recognizes methyllysine but does not recognize lysine, and also presents a method for detecting methylated proteins by means of said anti-methyllysine antibody (in particular, refer to fig. 4 and 5).

(C)

Document 3 presents the Histone H3(di methyl K4) antibody; indicates that said Histone H3(di methyl K4) antibody is a polyclonal antibody; indicates that said Histone H3(di methyl K4) antibody specifically recognizes methylated lysine but does not recognize lysine; and further presents a method for detecting methylated proteins by means of said Histone H3(di methyl K4) antibody. Meanwhile, the document J. DOVER et al. (J. Biol. Chem., 2002, Vol. 277, pages 28368 to 28371), which was released in the year 2002, indicates that the Histone H3(di methyl K4) antibody was already being used.

(D)

Document 4 presents an antiserum (a polyclonal antibody) that is prepared by immunizing a guinea pig with proteins obtained by chemically methylating low density lipoproteins from guinea pigs (Met LDLs) (hereinafter referred to as invention 1 presented in document 4), and also presents a method for detecting methylated proteins by means of a system for detecting

the bonding of said polyclonal antibodies and the Met LDLs (in particular, refer to fig. 3). Meanwhile, the addition of methylated albumen, monomethyllysine or dimethyllysine to the system for detecting the abovementioned bonding will competitively inhibit the bonding of the antibodies and the Met LDLs, whereas lysine does not induce competitive inhibition; therefore, it is thought that the abovementioned polyclonal antibodies recognize methyllysine but do not recognize lysine. In addition, document 4 also indicates that if the same amount of monomethyllysine and dimethyllysine are added to the system for detecting the abovementioned bonding, the dimethyllysine will induce a higher level of competitive inhibition.

(E)

Document 4 presents an antiserum (a polyclonal antibody) that is prepared by immunizing a guinea pig with proteins obtained by chemically methylating albumin from guinea pigs (Me gp albumin) (hereinafter referred to as invention 2 presented in document 4), and presents a method for detecting methylated proteins by means of a system for detecting the bonding of said polyclonal antibodies and the Me gp albumin (in particular, refer to fig. 4). Meanwhile, the addition of methylated human albumin (Me h albumin) or dimethyllysine to the system for detecting the abovementioned bonding will competitively inhibit the bonding of the antibodies and the Me gp albumin, whereas lysine does not induce competitive inhibition; therefore, it is thought that the abovementioned polyclonal antibodies recognize methyllysine but do not recognize lysine. In addition,

document 4 indicates that if the same amount of monomethyllysine and dimethyllysine are added to the system for detecting the abovementioned bonding, the dimethyllysine will induce a higher level of competitive inhibition.

(1)

The inventions set forth in claims 1, 5 and 11 lack novelty in the light of document 1.

A comparison of the inventions set forth in claims 1, 5 and 11 and the invention disclosed in document 1 revealed that it is impossible to differentiate said inventions.

(2)

The inventions set forth in claims 1, 6 and 11 do not involve an inventive step in the light of document 1.

Document 1 also discloses the antigens that are used when preparing the anti-methyllysine antibody (Methyl(K4)H3) or the anti-methyllysine antibody (Methyl(K9)H3), and methods for preparing monoclonal antibodies against a given antigen by means of animals such as mice are well known. Such being the case, it would have been easy for a person skilled in the art to conceive of using the antigens disclosed in document 1 in order to prepare monoclonal antibodies that have properties similar to those of the anti-methyllysine antibody (Methyl(K4)H3) and the like. Furthermore, the effects that result from doing so cannot be considered to be significant.

(3)

The inventions set forth in claims 1, 6 and 11 lack novelty in the light of document 2.

A comparison of the inventions set forth in claims 1, 6 and 11 and the invention presented in document 2 revealed that it is impossible to differentiate said inventions.

(4)

The inventions set forth in claims 1, 5 and 11 lack novelty in the light of document 3.

A comparison of the inventions set forth in claims 1, 5 and 11 and the invention presented in document 3 revealed that it is impossible to differentiate said inventions.

(5)

The inventions set forth in claims 1, 6 and 11 do not involve an inventive step in the light of document 3.

Document 3 also presents the antigens that are used when preparing the Histone H3(di methyl K4) antibody. Such being the case, it would have been easy for a person skilled in the art to conceive of using the antigens presented in document 3 in order to prepare monoclonal antibodies that have properties similar to those of the Histone H3(di methyl K4) antibody. Furthermore, the effects that result from doing so cannot be considered to be significant.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

(6)

The inventions set forth in claims 1, 5 and 11 lack novelty in the light of document 4.

A comparison of the inventions set forth in claims 1, 5 and 11 and the invention presented in document 4 revealed that it is impossible to differentiate the polyclonal antibodies of the former inventions from the polyclonal antibodies of invention 1 and invention 2 presented in document 4; consequently, it is impossible to differentiate said inventions.

(7)

The inventions set forth in claims 1, 6 to 8 and 11 do not involve an inventive step in the light of document 4.

It would have been easy for a person skilled in the art to conceive of using methylated proteins like the Met LDLs and the Me gp albumin presented in document 4 in order to prepare monoclonal antibodies that have properties similar to those of the polyclonal antibodies of invention 1 and invention 2 presented in document 4. Furthermore, the effects that result from doing so cannot be considered to be significant.

(8)

The inventions set forth in claims 1, 6 to 8, 10 and 11 do not involve an inventive step in the light of document 4.

Document 4 is considered to pertain to the preparation of modified lysine residue-specific antibodies, which include a protein other than the immunity protein and are capable of reacting with

modified lysine residues (in particular, refer to the abstract). Meanwhile, methods for producing monoclonal antibodies by using the reaction characteristics of the antigens and the monoclonal antibodies as an index for selecting hybridomas which produce monoclonal antibodies that have desired reaction characteristics from among the various hybridomas obtained by immunizing an animal with an antigen such as a peptide. Such being the case, it would have been easy for a person skilled in the art to conceive of preparing modified lysine residue-specific monoclonal antibodies that include a protein other than the immunity protein and are capable of reacting with modified lysine residues by using the reaction characteristics of the monoclonal antibodies and a methyllysine-containing protein other than the immunity protein as an index for selecting hybridomas which produce the abovementioned monoclonal antibodies from among the various hybridomas obtained by immunizing an animal with a methylated protein like the Met LDLs and the Me gp albumen presented in document 4. Furthermore, the effects that result from doing so cannot be considered to be significant.

(9)

The inventions set forth in claims 1, 5, 9 and 11 do not involve an inventive step in the light of document 4.

Methods wherein antibody fractions that have desired reaction characteristics relative to an antigen such as a peptide are prepared from polyclonal antibodies by subjecting the antibodies to an affinity purification

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

technique that employs a column or the like with the antigen immobilized thereupon are well known. Such being the case, it would have been easy for a person skilled in the art to conceive of preparing modified lysine residue-specific polyclonal antibodies that include a protein other than the immunity protein and are capable of reacting with modified lysine residues by employing the reaction between the polyclonal antibodies and methyllysine or a methylated protein other than the immunity protein as an index while implementing an affinity purification technique in order to prepare antibody fractions from the polyclonal antibodies of invention 1 and invention 2 presented in document 4 or from the polyclonal antibodies prepared via immunization with a methylated protein in the manner presented in document 4. Furthermore, the effects that result from doing so cannot be considered to be significant.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box IV.3

Furthermore, both the feature wherein the abovementioned anti-methyllysine antibodies are "monoclonal antibodies" and the "method for detecting methylated proteins by means of said anti-methyllysine antibodies" are described in said document (in particular, refer to fig. 4 and 5).

Such being the case, the inventions set forth in claims 1 and 5 to 11 have been classified into three groups; i.e., a group corresponding to the general inventive concept of "anti-methyllysine antibodies that are polyclonal antibodies," a group corresponding to the general inventive concept of "monoclonal antibodies produced by hybridomas (which have a given property in common)," and a group corresponding to the general inventive concept of a "method for producing monoclonal antibodies by immunizing an animal with antigens obtained by chemically methylating a protein, and then selecting the hybridomas which secrete an antibody that recognizes a protein obtained by chemically methylating a protein other than said antigen from among the antibodies obtained in this manner."

As a result, the claims set forth three general inventive concepts that are associated with anti-methyllysine antibodies; however, there is no novel special technical feature that is common to all of these general inventive concepts. Consequently, the present international application is not considered to conform to the requirement of unity of invention (Rule 13 of the Regulations Under the PCT (Rule 13.1, 13.2 and 13.3)).

Supplemental Box

Box I.4

The amendment to claim 1, which adds the disclosure "is capable of reacting with the histones and Elongation factors 1α from animals," and the amendments to claims 9 and 10, which add the disclosure "foreign protein," are considered to go beyond the scope of the features set forth in the description, the claims and the drawings of the present international application as originally filed.

Examples 6 and 7 present antibodies capable of bonding to Elongation factor 1 and to the proteins from MOLT-4F (a human T-cell leukaemia cell line) that have a molecular weight of between 14.4k and 21.5k. However, is not considered to be obvious to a person skilled in the art that said antibodies will also be capable of reacting with human histones, with histones from animals other than humans, and with Elongation factors 1α from animals other than humans. Furthermore, the description (paragraph [0013] and the examples) indicates that mice and rabbits are immunized with proteins such as a methylated KLH protein. However, is not considered to be obvious to a person skilled in the art that it is possible to immunize an animal with a protein from an animal of a species different from that of the animal to be immunized (i.e., with a foreign protein).